

Mechanisms of Cyclosporine A Inhibition of Antigen-Presenting Activity in Uninvolved and Lesional Psoriatic Epidermis

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To elucidate how cyclosporine A affects antigen-presenting cell subsets and their function in human skin, we studied patients with psoriasis undergoing a therapeutic trial of cyclosporine A. Immunologic parameters abnormal in psoriatic epidermis were evaluated before and early in the course of therapy. We quantitated function and numbers of skin biopsy-derived epidermal cells with potential antigen-presenting cell (APC) activity. The antigen-presenting capacity of epidermal cells from normal-appearing skin to activate allogeneic T cells was profoundly inhibited (81% decrease) 7 d after the onset of therapy ($p < 0.05$). Thus, cyclosporine A therapy inhibited T-cell activation mediated by Langerhans cells in uninvolved skin. By contrast, in lesional skin epidermal allo-antigen presenting activity was only partially inhibited at this early time point ($55 \pm 7\%$ decrease) ($p < 0.01$, $n = 8$). The percentage decrease in allo-antigen-presenting cell activity correlated with reduced clinical activity of the lesions, $r = 0.84$. In three patients also examined at 14 d, we found an additional $42 \pm 5\%$ decrease between day 7 and day 14. Decreased allo-antigen-presenting activity in lesional skin was not associated with a decrease in the number of CD1⁺ Langerhans cells or epidermal cell release of detectable amounts of cyclosporine A or other soluble factors that abrogate T-cell alloreactivity. The time course and degree of inhibition of antigen-presenting capacity

within involved psoriatic skin correlated best with a significant ($p < 0.01$) reduction in non-Langerhans cell DR⁺ leukocytes (from $3.0 \pm 1.2\%$ to $1.0 \pm 0.6\%$ at day 7) ($r = 0.71$). Cyclosporine A therapy was associated with a rapid and complete loss of HLe1-DR⁺ keratinocytes (94% decrease at 7 d) in lesional skin despite the skin still being quite involved with psoriasis at this point and antigen-presenting cell activity being only 60% reduced. In conclusion, cyclosporine A interferes with T-cell activation by human epidermis through at least two mechanisms: 1) in uninvolved skin, rapid inhibition of Langerhans cell-mediated activation of T cells, and 2) in lesional skin, delayed inhibition of antigen-presenting activity which appears to correlate with the time course and level of reductions in non-Langerhans cell DR⁺ leukocytes. The antigen-presenting activity of the latter cells appears to be cyclosporine A resistant. In psoriatic lesions, early and complete loss of DR expression on lesional keratinocytes during cyclosporine A therapy is likely due to decreased lesional T-cell lymphokine production critical for keratinocyte DR expression. Concomitantly, decreased recruitment and retention of infiltrating leukocytes by T-cell lymphokines may result in the depletion of non-Langerhans antigen-presenting leukocytes from lesional epidermis. *J Invest Dermatol* 94:649-656, 1990

Psoriasis is a multifactorial inflammatory disease of controversial etiology. Several self-perpetuating mechanisms of inflammation occur in concert with cellular hyperproliferation [1]. There is strong evidence for an increased population of proliferating keratinocytes and

for a markedly decreased transit time of keratinocytes within the epidermis [2,3]. However, it is not clear whether a primary epidermal defect causes elaboration of cytokines that result in increased vascularity, in dermal and epidermal infiltration with leukocytes, in altered eicosanoid profiles, and in fibroblast alterations; or whether epidermal hyperproliferation occurs as a phenomenon secondary to inductive dermal influences [4,5]. Indeed, careful histologic examination of early lesions implicates activation of lymphocytes, endothelial cells, mast cells, and macrophages preceding epidermal proliferation [4,6].

The successful use of cyclosporine A to treat psoriasis in controlled trials [7,8] and open studies [9-14] provide support for the concept that immune cellular activation in the lesion [15-18] might play an important role in the inflammation and epidermal hyperproliferation of psoriasis. Although phenotypic immunologic marker studies had identified the presence of increased numbers of lesional T cells, macrophages, and other non-Langerhans DR⁺ cells [7,17,19-23], we have recently demonstrated an increased functional capacity of immunocompetent cells in psoriasis skin [18,22]. Thus, there is an increased alloantigen-presenting capacity of in-

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Abbreviations:

- APC: antigen-presenting cells
- PBS: phosphate-buffered saline
- MNC: mononuclear cells
- MHC: major histocompatibility complex
- UN-PS: uninvolved psoriatic skin
- IN-PS: involved psoriatic skin

volved epidermis [18], an increased capacity to activate autologous blood T cells [24], and the presence of autoreactive T cells in lesional psoriasis dermis that can proliferate and release lymphokines in response to autologous lesional epidermal cells [25]. Because activated T cells release gamma interferon, and because gamma interferon is currently the only lymphokine known to induce DR on keratinocytes [26–29], the concept that such T-cell activation is indeed occurring in psoriasis lesions is supported by the observation of DR expression on psoriasis epidermal keratinocytes [15,16,18] and the expression by psoriatic keratinocytes of IP10, another gamma interferon-inducible protein [30]. In addition, psoriatic basal keratinocytes express UM4D4 [31], a molecule inducible on keratinocytes by exposure to lymphokines from lesional T-cell clones [32]. The clinical observation that psoriasis worsens in association with microbial infections (group A streptococci, AIDS [33], other viruses) also implicates leukocyte activation in providing a positive signal for the development of psoriasis.

Cyclosporine A exerts inhibitory effects on T-cell activation [34] and antigen-presenting cell function in vitro [35–40] and therapy of psoriasis results in clearing of T cells and other T-dependent immunocompetent cells from the lesion [7,22,41,42]. However, cyclosporine A has complex effects on T cells in vivo. Inhibition of T-cell responses is dependent upon the T-cell subset involved [43], the antigenic system employed [44,45], and the activation stage of the T cells [46]. In addition, cyclosporine A may exert direct effects on a variety of nonimmunologic cells, including keratinocytes [7,47,48], endothelial cells [49], kidney epithelial cells [50], and neutrophils [51,52].

To elucidate whether cyclosporine A may improve psoriasis on an immunologic basis, we examined patients' skin at the earliest time points when clinical responses begin to occur. We performed assays to determine whether cyclosporine A altered the proportions and function of epidermal antigen-presenting cells in the involved and uninvolved epidermis of psoriasis, and quantified changes in keratinocyte expression of DR (likely an *in vivo* bioassay of recent gamma-interferon release by *in situ* T cells). We observed a rapid decrease in antigen-presenting cell activity of uninvolved epidermis and a delayed but similar effect on involved epidermis. In concert with the loss of non-Langerhans cell antigen-presenting cells and disappearance of DR⁺ keratinocytes, these data indicate that cyclosporine A may interfere with lesional T-cell activation not only through its direct effects on T-cell lymphokine release, but also through effects on the ability of Langerhans cells in normal epidermis to activate T cells and on skin antigen-presenting cell subset numbers.

MATERIALS AND METHODS

Patients Male patients with severe psoriasis were treated with cyclosporine A (Sandimmune, Sandoz, Hanover, NJ) at a dose of 14 mg/kg/d for four weeks. Their clinical characteristics, side effects, and clinical responses have been previously reported [7]. Clinical activity scores used in this report for correlations represented the sum of mean erythema, thickness, and scaling scores from three indicator lesions followed over time. The protocol and consent form had been approved by The University of Michigan Medical Center Institutional Review Board. All patients gave informed consent after the nature of the study had been fully explained.

Preparation of Cell Suspensions Six-millimeter punch biopsies were obtained prior to therapy and following either 3 and 7 d or 7 and 14 d of cyclosporine A treatment from the originally biopsied plaque (involved) or an area adjacent to the original biopsy of clinically normal appearing skin (uninvolved). Biopsies were taken in the hip or buttock area. Uninvolved skin represents an area without psoriasis which was bordered on each side by at least 3 cm of normal appearing skin. Biopsies were incubated in Dulbecco's phosphate-buffered saline (PBS) containing 0.25% trypsin for 14 h at 4°C. Epidermal sheets were removed from the dermis and a fine epidermal cell suspension was obtained as previously described [18]. Allogeneic peripheral blood mononuclear cells (MNC) were isolated

from heparinized venous blood by Ficoll Hypaque density gradient centrifugation. *In vitro* allogeneic epidermal cell-lymphocyte reactions were performed in round bottom microtiter wells in 0.2 ml RPMI 1640 supplemented with 10% AB serum, glutamine, penicillin, and streptomycin. Gamma-irradiated (2000 rad) epidermal cells were added in varying numbers to 50,000 allogeneic responder MNC. The cultures were pulsed after 6 d with 1 μ Ci of ³[H]-TdR per well, harvested 18 h later on a semiautomated cell harvester (PHD Cambridge Technology, Inc., Cambridge, MA), and ³H-TdR incorporation was measured on a Packard Scintillation Counter (Packard Instrument Company, Downers Grove, IL). Results are expressed as mean \pm SEM of triplicate wells. The same allogeneic donor (KDC) was used throughout the study as a source of responder MNC to assess the stimulatory capacity of pre- and post-therapy epidermal cells. Positive controls were performed utilizing phytohemagglutinin (PHA).

In certain experiments, epidermal cells were obtained from involved psoriasis skin 7 d following therapy with cyclosporine A (14 mg/kg/d) and placed in culture in flat-bottom 24-well macro plates (Costar) at 1×10^6 per ml for 48 h, and the supernatant harvested after centrifugation. In order to determine whether epidermal cells obtained from cyclosporine A-treated patients were releasing cyclosporine A into the culture media that could then inhibit T-cell activation, the cyclosporine A content of the supernatants was determined using high-pressure liquid chromatography analysis as previously reported [7]. The supernatant was then added to an allogeneic epidermal cell-lymphocyte reaction consisting of epidermal cells autologous to the cells which produced the supernatant along with allogeneic responding mononuclear cells. The supernatant was added into the reaction mixture at a dilution of 1:4 or 1:16 and the effect was compared with cyclosporine A (1 μ g/ml).

To determine whether the maximum possible concentration of cyclosporine A released by epidermal cells *in vitro* into supernatants would inhibit T-cell activation, cyclosporine A dissolved in ethanol, or ethanol alone was diluted in PBS, and added in various concentrations to MNC and phytohemagglutinin or to a two-way mixed leukocyte reaction. Results were determined as cpm ³[H]-TdR uptake over the last 18 h of culture.

Cell-Surface Staining of Epidermal Cells in Suspension

Cell suspensions were double stained simultaneously with fluorescein-conjugated OKT6 (Ortho Diagnostic Systems, Raritan, NJ) or fluorescein-conjugated HLe1 (Becton Dickinson Monoclonal Center, Mountainview, CA) and biotin-conjugated anti-HLA-DR (Becton Dickinson Monoclonal Center) antibodies or isotype controls for 45 min at 4°C. The cells were washed and a second incubation was performed using streptavidin conjugated with Texas Red (BRL/Gibco, Bethesda, MD) [18,53]. A Nikon fluorescence microscope with appropriate filters was used for scoring cells labeled with one or two colors as previously described [53]. Results are expressed as a paired group mean percent \pm SEM of viable positive stained cells relative to total cell count.

Antibody Characteristics OKT6 is directed against an epitope on the CD1 determinant on Langerhans cells and immature thymocytes. Anti-HLA-DR is a monoclonal antibody directed against a non-polymorphic epitope on the HLA-DR human Class II major histocompatibility complex (MHC) antigen. Anti-HLe-1 is a monoclonal antibody which reacts with a determinant on the CD45 complex expressed on all bone marrow-derived leukocytes.

Statistical Evaluation Following statistical consultation, Wilcoxon rank sum analyses on paired data and regression analyses were performed and p values reported from standard tables.

RESULTS

Decreased Antigen-Presenting Capacity of Epidermis from Cyclosporine A-Treated Psoriasis Patients To determine whether systemic therapy with cyclosporine A altered the functional activity of immunocompetent cells within human epidermis, epidermal cells were obtained before and following 7 d of cyclo-

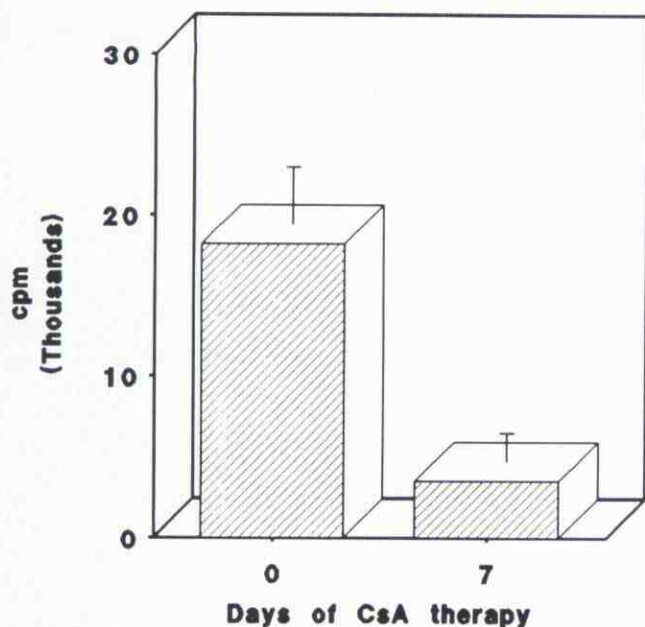


Figure 1. Stimulation of 50,000 allogeneic MNL by 30,000 psoriatic epidermal cells from clinically normal appearing skin before and after 7 d of cyclosporine A therapy. $n = 6$ patients, paired data. Results are mean \pm SEM cpm ^3H -TdR uptake.

sporine A therapy. 50,000 allogeneic peripheral blood MNC were mixed with varying concentrations of uninvolved epidermal cells and proliferation recorded as mean \pm SEM cpm ^3H -TdR uptake (Fig 1). There was diminished alloantigen-presenting cell capacity in the epidermal cells from clinically uninvolved skin of six of six patients with psoriasis (UN-PS) following 7 d of cyclosporine A therapy at all concentrations of epidermal cells tested; from a mean peak of $18,186 \pm 3,564$ cpm pretherapy to $3,539 \pm 1,764$ cpm after therapy ($p \leq 0.05$, $n = 6$, Fig 1). This reduction was apparent as early as 3 d following initiation of therapy and was more pronounced after 14 d of therapy (not shown).

In eight of eight patients tested, therapy with cyclosporine A also resulted in diminished antigen-presenting cell function by suboptimal as well as peak concentrations of epidermal cells from lesional, involved psoriatic skin. However, the inhibition occurred to a lesser extent than that in uninvolved skin at the 7-d time point. Utilizing 15,000 epidermal cells per well (which is the peak concentration using lesional epidermal cells as stimulators), mean paired values for eight patients were $31,089 \pm 4,348$ cpm pretherapy vs $16,716 \pm 4,472$ cpm after 7 d of therapy ($p \leq 0.01$, $n = 8$). Thus, while cy-

cyclosporine A therapy resulted in a profound 81% decrease in the antigen-presenting capacity of normal-appearing epidermis, therapy induced a partial decrease (mean $55 \pm 7\%$ decrease) in the stimulatory activity of involved psoriasis plaque epidermal cells at the 7-d time point. The plaques were still quite active at 1 week with the lesional activity scores of these patients demonstrating a $49 \pm 8\%$ decrease at 7 d. Allo-antigen presentation capacity at 7 d correlated with clinical lesion scores at $r = 0.84$, $p < 0.005$.

To help determine whether psoriatic epidermis is inherently resistant to cyclosporine A antigen-presenting cell activity effects or whether the residual antigen-presenting cell activity is attributable to the fact that at 7 d the skin is still quite involved with psoriasis, we examined antigen-presenting activity at day 14 as well as day 7 in three patients. In each of the three patients examined, 7 and 14 d after initiation of therapy, allo-antigen presentation demonstrated progressively decreased activity at both suboptimal (8,000) and peak (15,000) concentrations of epidermal cells (Table I). At 14 d, the mean decrease in peak antigen-presenting cell activity was $78 \pm 8\%$, representing an additional $42 \pm 5\%$ decrease between day 7 and day 14. Note, however, that the progressive diminution of antigen-presenting cell activity between day 7 and 14 was somewhat overcome in two patients by increasing the number of stimulating epidermal cells into the plateau/inhibitory concentration range (30,000) (Table I). This did not occur with uninvolved epidermis.

While Exerting No Effect on Langerhans Cell Numbers, Cyclosporine A Therapy Reduces Keratinocyte DR Expression and Depletes Infiltrating Non-Langerhans Cell DR⁺ Cells from Lesional Epidermis

Because antigen-presenting cell activity of involved psoriasis epidermis is critically dependent on Langerhans cells and non-Langerhans cell antigen-presenting cells [18], we quantitated changes in potential antigen-presenting cell subsets in the epidermal cell suspensions. In normal human epidermis, at least 90% of DR⁺ cells also express CD1, or T6, and these are defined as Langerhans cells [54]. In psoriasis epidermis, however, several types of DR⁺ cells with potential antigen-presenting cell activity are present. DR may be expressed on both bone marrow-derived cells (HLe-1⁺) and non-bone marrow-derived keratinocytes (HLe-1⁻) (Figs 3 and 4, day 0). Contained within the bone marrow-derived HLe-1⁺DR⁺ cells are both CD1⁺DR⁺ Langerhans cells and CD1⁻DR⁺ cells.

Following cyclosporine A therapy, Langerhans cells in lesional skin demonstrated no consistent or significant change in their percentage relative to total epidermal cells at 7 d (Fig 2), or at 14 d ($26 \pm 24\%$ decrease). In contrast, a large number of CD1⁻DR⁺ cells, which were abnormally present prior to therapy ($4.9 \pm 2.1\%$ on day 0), demonstrated a marked reduction in their percentage (to $0.8 \pm 0.3\%$) following 7 d of therapy ($p \leq 0.005$, $n = 7$) (Fig 2). Leukocytes not expressing DR were not significantly altered after 7 d of the treatment (Fig 3, HLe-1⁺DR⁻).

Table I. Antigen-Presenting Cell Activity of Involved Psoriatic Epidermis During Cyclosporine A Therapy

Patient	Day 0	Day 7	Day 14
8,000 epidermal cells ^a			
1	23,585 ^b	2,530	1,935
2	11,321	3,499	900
3	2,282	1,405	209
15,000 epidermal cells ^a			
1	27,505	10,220	5,023
2	24,118	13,930	9,127
3	7,475	1,331	814
30,000 epidermal cells ^a			
1	20,930	18,371	15,638
2	23,262	11,296	13,552
3	5,469	5,124	2,302

^a 8,000, 15,000, or 30,000 epidermal cells were mixed with 50,000 MNL for 7 d.

^b CPM (triplicate mean) of tritiated thymidine uptake by proliferating lymphocytes over the last 18 h of culture.

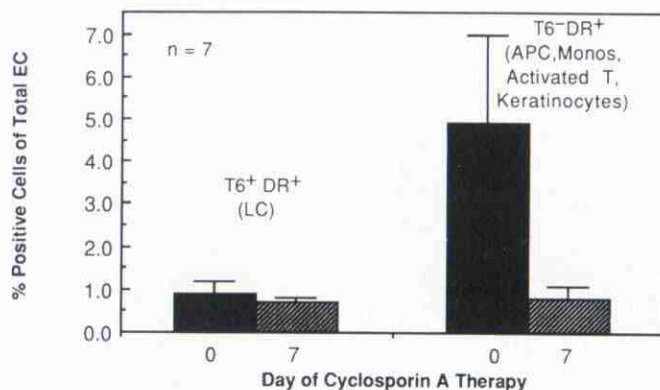


Figure 2. Percentage of T6⁺DR⁺ Langerhans cells and T6⁻DR⁺ epidermal cells in involved psoriatic skin before and following 7 d of cyclosporine A treatment. Results are expressed as percentage of total epidermal cells \pm SEM. Group means of seven patients, paired data.

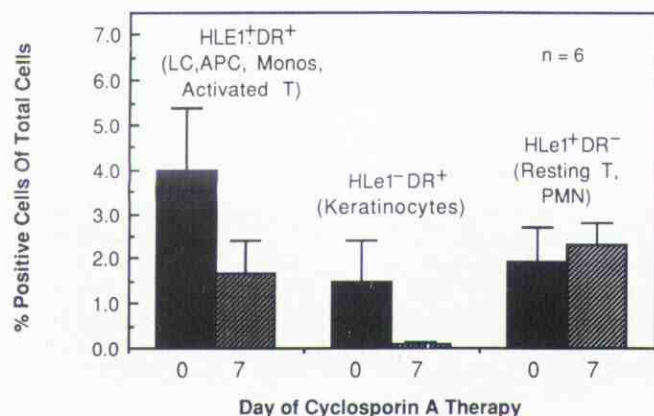


Figure 3. Percentage of HLE1⁺DR⁺, HLE1⁻DR⁺, and HLE1⁺DR⁻ EC in involved psoriasis skin before and following 7 d of cyclosporine A treatment. Results are expressed as percentage of total epidermal cells \pm SEM. Group means of six patients, paired data.

CD1⁺DR⁺ lesional epidermal cells in psoriatic plaques contained both non-bone marrow-derived DR⁺ keratinocytes (HLE1⁻DR⁺) and bone marrow-derived DR⁺ cells distinct from Langerhans cells (HLE1⁺DR⁺ subtracted by the number of CD1⁺DR⁺) (non-Langerhans cell DR⁺ leukocytes) (Figs 2–4). We therefore examined the response of each of these populations to cyclosporine A therapy. Cyclosporine A therapy induced a dramatic, fifteenfold (94%) decrease in DR⁺ keratinocytes by day 7 (Fig 3). In a paired comparison of the same six patients at the same time point, non-Langerhans cell DR⁺ leukocytes demonstrated only a threefold (67%) decrease, from 3.1% of epidermal cells prior to therapy to 1.0% of epidermal cells after 7 d of therapy (non-Langerhans cell DR⁺ leukocytes decreased in eight of eight patients tested, $p < 0.01$). To examine the full time course of depletion of non-Langerhans cell DR⁺ leukocytes over 14 d of therapy, data from all eight patients were analyzed as percentage of day 0 values to normalize variations in day 0 percentages between patients. Progressive diminution in the number of non-Langerhans cell bone marrow-derived DR⁺ cells oc-

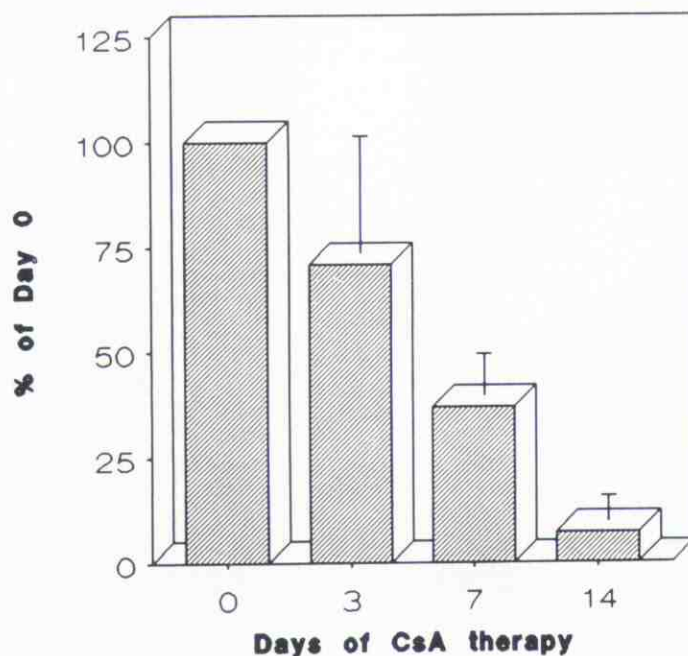


Figure 4. Effect of cyclosporine A on non-Langerhans antigen-presenting leukocytes (HLE1⁺DR⁺ — CD1⁺DR⁺) in lesional psoriatic epidermis. Raw values normalized to 100% on day 0, unpaired data. Day 0, $n = 7$; day 3, $n = 3$; day 7, $n = 6$; day 14, $n = 3$.

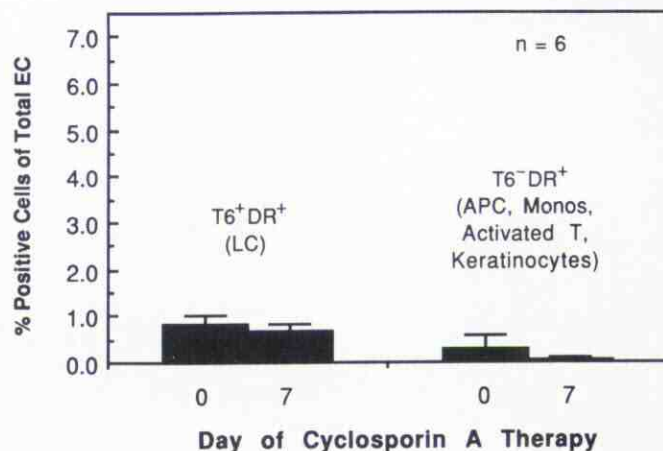


Figure 5. Percentage of T6⁺DR⁺ LC and T6⁻DR⁺ EC in uninvolved psoriasis skin before and following cyclosporine A treatment. Results are expressed as percentage of total EC \pm SEM. Group means of six patients, paired data.

curred between day 3 and day 14 (Fig 4) in a manner that corresponds well to the time course of inhibition of antigen-presenting cell activity and clinical clearing in this group of patients. The mean decrease (unpaired data) in antigen-presenting cell activity at day 3 was -4.4% ; at day 7, 55% ; at day 14, 78% . The mean decrease (unpaired data) in non-Langerhans cells DR⁺ leukocytes at day 3 was 29% ; at day 7, 63% ; at day 14, 93% . For comparison, the clinical lesional activity scores in these same eight patients demonstrated a mean decrease at day 3 of $30 \pm 5\%$; at day 7, $49 \pm 8\%$; at day 14, $81 \pm 4\%$. While changes in CD1⁺DR⁺ Langerhans cells and HLE1⁻DR⁺ keratinocytes correlated poorly with alloreactivity ($r = 0.0$), changes in alloreactivity correlated strongly with non-Langerhans cell DR⁺ leukocyte levels at $r = 0.71$, $p < 0.005$.

Effect of Cyclosporine A on Immunocompetent Cells in Clinically Uninvolved Skin Loss of antigen-presenting cell activity by clinically uninvolved epidermis from psoriasis patients following cyclosporine A therapy could be due either to a direct inhibition of the functional capacity of Langerhans cells, or to depletion of antigen-presenting Langerhans cells. However, the percentage of CD1⁺DR⁺ Langerhans cells in normal skin showed only minimal change after therapy (from $0.9 \pm 0.2\%$ before to $0.7 \pm 0.1\%$ after therapy) (Fig 5) ($p = \text{NS}$, $n = 6$).

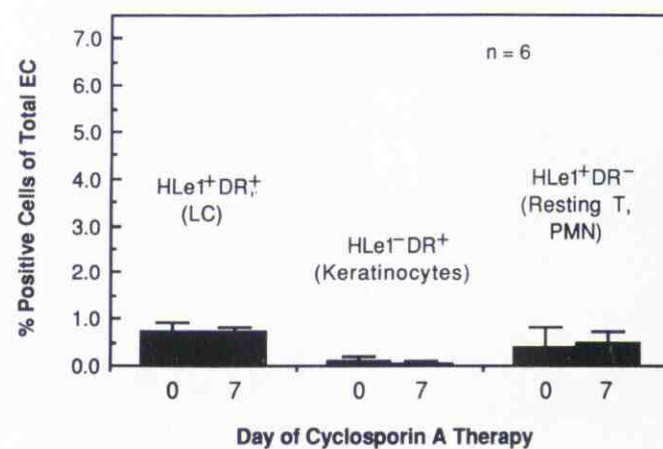


Figure 6. Percentage of HLE1⁺DR⁺, HLE⁻DR⁺, and HLE1⁺DR⁻ epidermal cells in uninvolved psoriasis skin before and following cyclosporine A treatment. Results are expressed as percentage of total epidermal cells \pm SEM. Group means of six patients, paired data.

In normal-appearing uninvolved skin of psoriasis patients, CD1⁺DR⁺ cells could be identified in five of seven patients, with a mean level of $0.3 \pm 0.1\%$ [18]. Although only representing a fraction of a percent of the epidermal population, no CD1⁺DR⁺ cells were observed following therapy with cyclosporine A therapy (Fig 5). In uninvolved psoriatic skin, both before and after therapy, the number of HLe1⁺DR⁺ cells closely approximated the number of CD1⁺DR⁺ cells, confirming that, as in normal skin, bone marrow-derived cells are comprised almost entirely of Langerhans cells. Again, no change was seen following therapy (Fig 6). DR⁺ keratinocytes (HLe1⁺DR⁺) were rarely observed in clinically uninvolved skin of psoriasis, both before and after therapy. Although HLe1⁺DR⁺ cells were present in the uninvolved skin of psoriasis patients, they were also unchanged with therapy (Fig 6). Thus, abrogation of alloantigen presentation activity in the epidermis of uninvolved skin of psoriasis patients appears not to be due to the disappearance of Langerhans cells or other antigen-presenting cells.

Decreased Stimulatory Capacity of Epidermis from Cyclosporine A-Treated Individuals Is Not Due to Inhibitory Factors (i.e., Cyclosporine A) Released In Vitro To address whether the diminished alloantigen-presenting capacity of epidermal cells is due to release of cyclosporine A or its metabolites into the media, we examined the inhibitory activity of epidermal cell supernatants. Epidermal cells were harvested from a patient treated for 2 weeks with cyclosporine A and placed in culture at 1×10^6 /ml for 48 h. The cell-free supernatant was then added to a reaction mixture consisting of 60,000 psoriasis epidermal cells, autologous to those used to generate the supernatant, in combination with 50,000 allogeneic MNC. Addition of the supernatant from treated psoriasis epidermal cells failed to abrogate allogeneic T-cell reactivity to psoriasis epidermal cells at both dilutions tested (1:4 and 1:16) (Table II). Addition of 250 ng/ml cyclosporine A, however, profoundly inhibited the response (Table II). Thus, soluble products of psoriasis epidermal cells harvested from involved skin during cyclosporine A therapy did not appear to inhibit T-cell proliferation in the epidermal lymphocyte reaction.

To further address the question of cyclosporine A release, epidermal cells of three patients from their involved and uninvolved skin before and after 14 d of cyclosporine A therapy were cultured for 48 h at $0.5-2 \times 10^6$ cells/ml. The supernatant was harvested, the lipid fraction extracted in ethanol and analyzed by HPLC for cyclosporine A concentration. No detectable cyclosporine A was found in any of the patient samples analyzed (assay detection limit of 5–10 ng/ml). Thus, because 2×10^6 /ml epidermal cells released less than 10 ng/ml cyclosporine A, and we used a 0.15×10^6 /ml epidermal cell concentration (30,000 epidermal cells in 0.2 ml per well) to stimulate T cells in our epidermal cell-lymphocyte reaction (a 13.3 times difference), then the maximum concentration of cyclosporine A released by epidermal cells in each well would be less than 0.75 ng/ml (10 ng/ml divided by 13.3).

Low concentrations of cyclosporine A in this range were added to PHA-stimulated MNC and to an allogeneic mixed leukocyte reac-

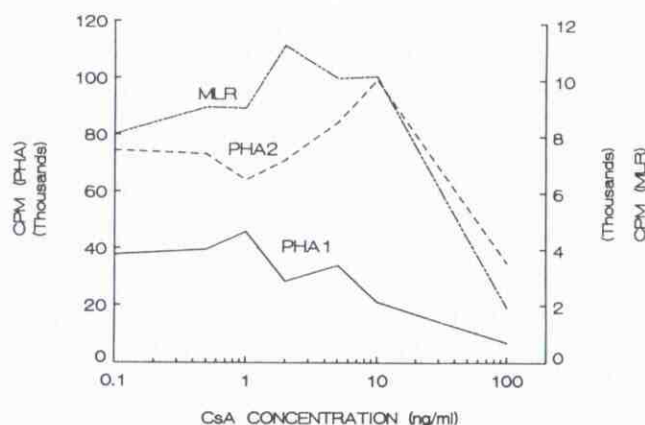


Figure 7. Cyclosporine A dose-response curves at low concentrations for inhibition of T-cell activation. Phytohemagglutinin (PHA) stimulated MNC from two individuals (A and B) and an allogeneic mixed leukocyte reaction (MLR) are demonstrated. The ethanol vehicle controls are identical to the 0 ng/ml cyclosporine A point.

tion to determine the degree of inhibition of T-cell activation by cyclosporine A under our conditions. Concentrations in the 100-ng/ml range were required to significantly inhibit the T-cell responses (Fig 7). This concentration is well beyond that potentially released by cultured epidermal cells.

DISCUSSION

We report observations on cellular immune reactivity of cells obtained from the skin of patients with psoriasis before and early during treatment with cyclosporine A. We examined both lesional skin as well as normal-appearing skin of patients during the time when clinical responses could first be detected. We have previously shown that lesional psoriasis epidermis contained an increased percentage of infiltrating non-Langerhans cell leukocytes that express HLA-DR, and that these leukocytes, but not DR⁺ keratinocytes, are responsible for the increased antigen-presenting cell activity of psoriatic epidermis [18]. The increased capacity of lesional psoriatic epidermis to stimulate autologous T cells [24] and the identification of autoreactive T-cell clones within psoriasis skin [25] suggested antigen-presenting cells and their activity may be important in the pathophysiology of psoriatic lesions. We thus focused our examination on antigen-presenting cells, their functional activity, and HLe1⁺DR⁺ keratinocytes (a putative *in vivo* marker of lesional T-cell lymphokine release [26]) to help identify the mechanisms by which cyclosporine A may clear psoriasis.

In this study we show that lesional infiltrating non-Langerhans cell DR⁺ leukocytes, but not Langerhans cells themselves, are reduced early in the course of cyclosporine A treatment. This decrease occurred in concert with the dramatic loss (94% decrease at 7 d) of DR expression by keratinocytes, suggesting that decreased T-cell lymphokine release and retention of non-Langerhans cell DR⁺ leukocytes in the epidermis are linked events. Concentrations of 2000 ng/ml cyclosporine A were achieved in the lesional skin of these patients [45], a concentration sufficient to inhibit intraepidermal T-cell activation (Fig 7). Thus, decreased T-cell activation during cyclosporine A treatment likely occurs at least in part by inhibition of molecular events of T cell activation [34,51]. However, decreased T-cell activation may also be due to effects on the antigen-presenting cell activity of the epidermis [35–40,55]. Clinically normal-appearing psoriasis skin, while showing no change in Langerhans cell numbers (Fig 5), showed a substantial 81% decrease in its capacity to activate T cells. Epidermal cells from clinically involved psoriasis plaques also showed decreased antigen-presenting cell activity, but the effect appears more delayed.

There are three major possibilities that could explain the decreased antigen-presenting cell activity of epidermis following sys-

Table II. Effect of Psoriasis Epidermal Cell Supernatant on Epidermal Cell-Lymphocyte Reaction

R ^a	S ^b	Sup ^c	cpm ^d	SEM
MNC			528	153
MNC	EC		33459	4647
MNC	EC	post CsA EC sup (1:16)	30890	5305
MNC	EC	post CsA EC sup (1:4)	25863	8924
MNC	EC	CsA 250 ng/ml	5901	1415

^a R = responder cells (50,000/well) allogeneic to stimulator epidermal cells (EC).

^b S = stimulator epidermal cells (gamma irradiated) (60,000/well) from psoriasis involved skin.

^c Sup = supernatant of epidermal cells obtained from psoriasis involved skin after 14 d of therapy with CsA at 14 mg/kg/d. EC were cultured at 1×10^6 /ml for 48 h prior to supernatant harvest.

^d Results expressed as mean cpm \pm SEM of ³H-TdR uptake over the last 18 h of culture.

temic therapy with cyclosporine A: 1) a direct cyclosporine A effect on antigen-presenting function, 2) complex alterations in the balance of inhibitory and supportive factors released into the supernatant by cyclosporine A-treated epidermal cells, and 3) changes in numbers of cells that are critically involved in alloantigen presentation.

A direct effect on antigen-presenting cell presentation function is best supported by our results from clinically normal-appearing skin, in which, despite unchanged Langerhans cell numbers, a marked decrease in alloantigen presentation occurred (Fig 1). Although cyclosporine A, in general, had limited effects on macrophage immune effector functions, several reports have suggested that cyclosporine A may interfere with antigen-presentation function [35–40,55]. Furue and Katz have shown that, while *in vitro* loading of Langerhans cells with cyclosporine A appears to result in a direct inhibition of murine Langerhans cell antigen presentation, epidermal cells from systemically treated animals released cyclosporine or other mediators that were inhibitory for T cells [36].

In our human study, less than 0.75 ng cyclosporine A/ml was released by treated epidermal cells; 100 ng/ml was necessary to begin to inhibit T-cell proliferation (Fig 7). Our inability to demonstrate that supernatant from the cells of treated patients could abrogate T-cell responses also mitigates the likelihood that significant cyclosporine A release accounts for the inhibition of the allogeneic epidermal cell-lymphocyte reaction (Fig 1). However, because dendritic antigen presenting cells exhibit extensive membrane contact with T cells during T-cell activation, a "direct" effect of cyclosporine A on Langerhans cells may result through the transfer of cyclosporine A to the T cell from the Langerhans cell. Keratinocytes or other cells might also contain sufficient cyclosporine A on their surface membrane to carry an inhibitory effect into a coculture with T cells. If this mechanism were dominant, one would predict that increasing concentrations of epidermal cells would result in decreasing T-cell activation. In the case of lesional psoriasis, the opposite was true; concentrations of epidermal cells which were in the plateau/inhibitory range (30,000) relative to the peak concentration (15,000) on day 0 (Table I), were more stimulatory than lower concentrations when obtained from cyclosporine A-treated lesions (Table I). These results are more consistent with our findings that residual non-Langerhans DR⁺ leukocytic antigen-presenting cells are still present to some degree within the incompletely cleared psoriatic lesions at 7 d.

Indeed, changes in cell numbers critical for antigen-presenting cell activity by lesional psoriatic epidermal cells appear highly important in the degree of T-cell proliferation observed. Antigen-presenting cell activity of lesional psoriasis epidermal cells is totally dependent upon DR⁺ leukocytes; DR⁺ keratinocytes are not activating in this assay [18]. Therefore, the complete loss of DR⁺ keratinocytes would not be expected to alter the epidermal antigen-presenting cell activity. Langerhans cells themselves are unchanged in number with therapy (Fig 2); furthermore they contribute minimally if at all to the antigen-presenting cell activity of untreated lesional epidermal cells [18]. However, the partial (67%) decrease in the non-Langerhans cell antigen-presenting cells at day 7 (Fig 4) correlates well with the partial (55%) decrease in antigen-presenting cell activity of involved psoriasis epidermis at that time point. Their further depletion by day 14 (Fig 4) correlates again with progressively decreased antigen-presenting cell activity at day 14 (Table I). Potential antigen-presenting cells in the non-Langerhans cell antigen-presenting cell population include Mono1⁺ monocytes [22,42] as well as dendritic antigen-presenting cells expressing RFD1 [7,56,57]. Cells within both the epidermis and dermis expressing such markers are reduced in density in histologic sections following cyclosporine A therapy [7,42]. If these residual non-Langerhans cell DR⁺ leukocytes are responsible for the residual APC activity, it is not clear why they, in contrast to Langerhans cells in uninvolved skin, would not be inhibited in their functional activity by cyclosporine A. Because they are distinct from Langerhans cells, their binding of, sensitivity to, or release of cyclosporine A may be less than that of Langerhans cells. Whether Langerhans cell-me-

diated antigen presentation in lesional psoriatic skin is inhibited during CsA therapy is unclear from our studies. However, the levels of alloreactivity at 14 d (when non-Langerhans antigen presenting cell numbers are low) using 8×10^3 and 15×10^3 epidermal cells are somewhat less than we usually observe using epidermal cells from normal skin at these concentrations (Table 1).

The decreased sensitivity of lesional antigen-presenting cells to direct effects of cyclosporine A cannot be attributed to lower concentrations of cyclosporine A in lesional psoriatic skin than in uninvolved skin; in fact, significantly lower concentrations of cyclosporine A were achieved in uninvolved skin (500 ± 100 pg/mg DNA, $n = 6$) than in lesional psoriatic skin (900 ± 100 pg/mg DNA, $n = 10$) on day 7 [47]. Nor does it seem likely that progressive diminution of antigen-presenting cell activity can be related to increasing cyclosporine A tissue levels, since these levels are mostly achieved by day 3 (700 ± 100 pg/ μ g DNA on day 3 vs 900 ± 100 pg/ μ g DNA on day 7, $p = 0.19$) [47]. It thus may be likely that different mechanisms of antigen-presenting cell inhibition occur in uninvolved and lesional skin during cyclosporine A therapy. Decreased antigen-presenting cell activity of normal skin may be due to a direct Langerhans cell functional effect or carryover of cyclosporine A (Fig 1) [36], but not to an effect on Langerhans cell number or expression of CD1 or HLA-DR (Figs 2 and 5). The time course and level of decreased antigen-presenting cell activity in lesional skin during cyclosporine A therapy appears most correlated with numbers of non-Langerhans cells DR⁺ leukocytic antigen-presenting cells, which do not appear to be directly inhibited by cyclosporine A, even at concentrations of cyclosporine A twice that of uninvolved skin. By contrast, inhibited antigen-presenting cell activity in lesions correlated poorly with numbers of DR⁺ keratinocytes and Langerhans cells.

By looking early in the course of treatment, we were able to identify selective events and changes in epidermal cells relevant for antigen presentation. Signals that retain Langerhans cells in the epidermis are unaffected by cyclosporine (Figs 2 and 5). The rapid decrease in the expression of DR by keratinocytes suggested a rapid turnoff of gamma-interferon release by lesional activated T cells. Simultaneously, a non-Langerhans cell bone marrow-derived DR⁺ leukocyte population that contained various antigen-presenting cell subtypes was also disappearing from the epidermis, in concert with decreasing antigen-presenting cell activity. Decreased recruitment of these cells either through decreased lymphokine production by activated T cells or through a reduction in chemotactic eicosanoids is possible, as is a direct effect of cyclosporine A on these cells.

That the effect of cyclosporine was selective and did not induce the loss of all infiltrating immunologic cell types was evident by our finding that not only Langerhans cells, but also the HLe1⁺DR⁺ population, was unchanged in relation to the total population despite 7 d of cyclosporine A therapy (Fig 4). Since our histologic studies demonstrated a marked reduction in the number of polymorphonuclear leukocytes in the epidermis following cyclosporine A therapy [7], these data suggest that the residual cells were comprised of T cells which were not expressing the HLA-DR activation marker.

Heightened immunologic reactivity in lesional psoriasis skin appears to be related to increased antigen-presenting cell activity and T-cell activation, either as a form of autoimmunity or as a response to some unknown antigenic or mitogenic stimulus. Lymphokine production by such activated T cells is likely to be involved directly in the recruitment and activation of additional inflammatory leukocytes, and to induce keratinocytes to express ICAM-1 [58] and HLA-DR [15]. In fact, we have found that T-cells derived from lesional psoriasis skin release lymphokines that directly stimulate keratinocytes to express ICAM-1, HLA-DR, and the keratinocyte activation marker, UM4D4 [32]. Cyclosporine A may interfere with several events in this cascade. Cyclosporine A has direct effects on the molecular events of T-cell activation and lymphokine release, and may have additional effects on antigen-presenting cell function and recruitment in the epidermis, thus providing a multistage inhibition of T-cell activation. Further effects of cyclosporine

A on arachidonic acid metabolism, cytokine production, and activation status of keratinocytes may act synergistically to interfere with multiple events in the amplifying cascades of inflammation involved in the pathogenesis of psoriasis.

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REFERENCES

- Steigleder GK: Basic pathogenetic aspects of psoriasis. In: *Psoriasis: Proceedings of the Third International Symposium*. Cox AJ (ed.). Grune & Stratton, New York, 1982, p 13-22
- Weinstein GD, McCullough JL, Ross PA: Cell kinetic basis for pathophysiology of psoriasis. *J Invest Dermatol* 85:579-583, 1985
- Weinstein GD, Frost P: Cell proliferation kinetics in benign and malignant skin diseases in humans. *Natl Cancer Inst Monogr* 30:225-246, 1969
- Braverman IM, Sibley J: Role of the microcirculation in the treatment and pathogenesis of psoriasis. *J Invest Dermatol* 78:12-17, 1982
- Saia P, Coulomb B, Lebreton C, Bell E, Dubertret L: Psoriatic fibroblasts induce hyperproliferation of normal keratinocytes in a skin equivalent model in vitro. *Science* 230:669-672, 1985
- Schubert C, Christophers E: Mast cells and macrophages in early relapsing psoriasis. *Arch Dermatol* 277:352-358, 1985
- Ellis CN, Gorsulowsky DC, Hamilton TA, Headington JT, Cooper KD, Baadsgaard O, Duell EA, Annesley TM, Turcotte JG, Voorhees JJ: Cyclosporin A improves psoriasis in a double blind study. *JAMA* 256:3110-3116, 1986
- Van Joost T, Bos JD, Heule F, Meinardi MM: Low-dose cyclosporine A in severe psoriasis. A double-blind study. *Br J Dermatol* 118:183-190, 1988
- Van Joost T, Heule F, Stolz E, Beukers R: Short-term use of cyclosporine A in severe psoriasis. *Br J Dermatol* 114:615-620, 1986
- Harper JI, Keat AC, Staughton RC: Cyclosporin for psoriasis. *Lancet* II:981-982, 1984
- van Hooff JP, Leunissen KM, Staak W: Cyclosporine and psoriasis. *Lancet* I:335, 1985
- Marks JM: Cyclosporine A treatment of severe psoriasis. *Br J Dermatol* 115:745-746, 1986
- Griffiths CE, Powles AV, Leonard JN, Fry L, Baker BS, Valdimarsson H: Clearance of psoriasis with low dose cyclosporin. *Br Med J Clin Res* 293:731-732, 1986
- Picascia DD, Garden JM, Freinkel RK, Roenigk HH: Treatment of resistant severe psoriasis with systemic cyclosporine. *J Am Acad Dermatol* 17:408-414, 1987
- Morhenn VB, Abel EA, Mahrle G: Expression of HLA-DR antigen in skin from patients with psoriasis. *J Invest Dermatol* 78:165-168, 1982
- Gottlieb AB, Lifschitz B, Fu SM, Staiano-Coico L, Wang CY, Carter DM: Expression of HLA-DR molecules by keratinocytes, and presence of Langerhans cells in the dermal infiltrate of active psoriatic plaques. *J Exp Med* 164:1013-1028, 1986
- Baker BS, Swain AF, Fry L, Valdimarsson H: Epidermal T lymphocytes and HLA-DR expression in psoriasis. *Br J Dermatol* 110:555-564, 1984
- Baadsgaard O, Gupta AK, Billings A, Brown M, Ellis C, Voorhees JJ, Cooper KD: Psoriasis epidermal cells demonstrate increased number and function of non-Langerhans cell antigen presenting cells. *J Invest Dermatol* 92:190-195, 1989
- Baker BS, Swain AF, Griffiths CEM, Leonard JN, Fry L, Valdimarsson H: Epidermal T lymphocytes and dendritic cells in chronic plaque psoriasis: the effects of PUVA treatment. *Clin Exp Immunol* 61:526-534, 1985
- Bjerke JR: In situ characterization and counting of mononuclear cells in lesions of different clinical forms of psoriasis. *Acta Dermatol Venereol* 62:93-100, 1981
- Bos JD, Huslebosch HJ, Krieg SR, Bakker PM, Cormane RH: Immunocompetent cells in psoriasis: in situ immunophenotyping by monoclonal antibodies. *Arch Dermatol Res* 275:181-189, 1983
- Stingl G, Wolff K, Diem E, Baumgartner G, Knapp W: In situ identification of lymphoreticular cells in benign and malignant infiltrates by membrane receptor sites. *J Invest Dermatol* 69:231-235, 1977
- Bos JD, van Garderen ID, Krieg SR, et al: Different in situ distribution patterns of dendritic cells having Langerhans (T6+) and interdigitating (RFD 1+) cell immunophenotype in psoriasis, atopic dermatitis and other inflammatory dermatoses. *J Invest Dermatol* 87:358, 1986
- Schopf RE, Hoffman A, Jung M, Morsches B, Bork K: Stimulation of T cells by autologous mononuclear leukocytes and epidermal cells in psoriasis. *Arch Dermatol Res* 279:89-94, 1986
- Baadsgaard O, Hansen E, Chan L, Tong P, Wantzin GL, Voorhees JJ, Fox DA, Cooper KD: Lesional psoriasis T cell cultures reflect the in situ subset distribution and demonstrate autoreactivity (abstr). *J Invest Dermatol* 90:545, 1988
- Volc-Platzer B, Majdic O, Knapp W, Wolff K, Minterberger W, Lechner K, Stingl G: Evidence of HLA-DR antigen biosynthesis by human keratinocytes in disease. *J Exp Med* 159:1784-1749, 1984
- Basham TY, Nickoloff BJ, Merigan TC: Recombinant gamma interferon induces HLA-DR expression on cultured keratinocytes. *J Invest Dermatol* 83:88-92, 1984
- Czernielewski JM, Bagot M: Class II MHC antigen expression by human keratinocytes results from lympho-epidermal interactions and gamma interferon production. *Clin Exp Immunol* 66:295-302, 1986
- Nickoloff BJ, Fisher GJ, Mitra RS, et al: Additive and synergistic antiproliferative effects of cyclosporine A and gamma interferon on cultured human keratinocytes. *Am J Pathol* 131:12-18, 1988
- Gottlieb AB, Luster AD, Posnett DN, Carter DM: Detection of a gamma interferon-induced protein IP-10 in psoriatic plaques. *J Exp Med* 168:941-948, 1988
- Chan L, Taieb A, Fox DA, Marcelo C, Baadsgaard O, Cooper KD: Expression of UM4D4, a novel T cell activating molecule in normal skin; abnormal expression in psoriasis (abstr). *J Invest Dermatol* 90:551, 1988
- Baadsgaard O, Fisher G, Fox DA, Salvo B, Carey T, Marcelo C, Voorhees JJ, Cooper KD: Conditioned media from lesional psoriatic T-cell clones induces keratinocyte proliferation and expression of the surface activation molecule, UM4D4. *J Invest Dermatol* (in press)
- Staughton RC, Keat AC, Harper JI: Psoriasis, cyclosporine A, and AIDS. *Br Med J Clin Res* 294:125, 1988
- Shevach EM: The effects of cyclosporine A on the immune system. *Ann Rev Immunol* 3:397-423, 1985
- Knight SC, Roberts M, Macatonia SE, Edwards AJ: Blocking of acquisition and presentation of antigen by dendritic cells with cyclosporine. Studies with fluorescein isothiocyanate. *Transplantation* 46:48S-53S, 1988
- Furie M, Katz SI: The effect of cyclosporine on epidermal cells. I. Cyclosporine inhibits accessory cell functions of epidermal Langerhans cells in vitro. *J Immunol* 140:4139-4143, 1988
- Esa AH, Paxman DG, Noga SJ, Hess AD: Sensitivity of monocyte subpopulations to cyclosporine. Arachidonate metabolism and in vitro antigen presentation. *Transplantation Proc* 20:80-86, 1988
- Palay DA, Cluff CW, Wentworth PA, Ziegler HK: Cyclosporine inhibits macrophage-mediated antigen presentation. *J Immunol* 136:4348-4353, 1986
- Manca F, Kunkl A, Celada F: Inhibition of the accessory function of murine macrophages in vitro by cyclosporine. *Transplantation* 39:644-649, 1985
- Knight SC, Balfour B, O'Brien J, Buttifant L: Sensitivity of veiled (dendritic) cells to cyclosporine. *Transplantation* 41:96-100, 1986
- Baker BS, Griffiths CE, Lambert S, Powles AV, Leonard NJ, Valdimarsson H, Fry L: The effects of cyclosporine A on T lymphocyte and dendritic cell subpopulations in psoriasis. *Br J Dermatol* 116:503-510, 1987
- Gupta AK, Baadsgaard O, Brown M, Ellis C, Voorhees JJ, Cooper KD: Clearing of psoriasis with cyclosporine A is accompanied by reductions in the density of epidermal and dermal T cells and macrophages. *Arch Dermatol* (in press)
- Handsbumacher RE, Harding MW, Rice J, Drugge RJ, Speicher DW: Cyclophilin: A specific cytosolic bonding protein for cyclosporine A. *Science* 266:544-547, 1984

44. Black CD, Drocsek RA, Barbet J, Weinstein JN, Shevach EM: Induction of IL-2 receptor expression in vivo: response to concanavalin A. *Cell Immunol* 111:420-432, 1988
45. Krocsek RA, Black CD, Barbet J, Shevach EM: Mechanism of action of cyclosporine A in vivo. I. Cyclosporine A fails to inhibit T lymphocyte activation in response to alloantigens. *J Immunol* 139:3597-3603, 1987
46. Dos Reis GA, Shevach EM: Effect of cyclosporine A on T cell function in vitro: the mechanism of suppression of T cell proliferation depends on the nature of the T cell stimulus as well as the differentiation state of the responding T cell. *J Immunol* 129:2350-2367, 1982
47. Fisher GJ, Duell EA, Nickoloff BJ, Annesley TM, Kowalke JK, Ellis CN, Voorhees JJ: Levels of cyclosporine in epidermis of treated psoriasis patients differentially inhibit growth of keratinocytes cultured in serum free versus serum containing media. *J Invest Dermatol* 91:142-146, 1988
48. Furue M, Gaspari AA, Katz SI: The effect of cyclosporine A on epidermal cells. II. Cyclosporine A inhibits proliferation of normal and transformed keratinocytes. *J Invest Dermatol* 90:796-800, 1988
49. Zoja C, Furci L, Ghilardi F, Zilio P, Benigni A, Remuzzi G: Cyclosporine-induced endothelial cell injury. *Lab Invest* 55:455-462, 1986
50. Becker GM, Gandolofi AJ, Nagle RB: Effects of Cyclosporine A on a kidney epithelial cell line (LLC-PK). *Res Commun Chem Path Pharmacol* 56:277-280, 1987
51. Niwa Y, Kano T, Taniguchi S, Miyachi Y, Sakane T: Effect of cyclosporine A on the membrane-associated events in human leukocytes with special reference to the similarity with dexamethasone. *Biochem Pharmacol* 35:947-951, 1986
52. Pigatto PD, Mozzanica N, Polenghi MM, Altomare GF, Finzi AF: Cyclosporine A inhibits polymorphonuclear leukocyte chemotaxis in vivo. *Transplantation Proc* 20:91-94, 1988
53. Baardsgaard O, Cooper KD, Lisby S, Wulf C, Wantzin GL: Dose response and time course for induction of T6-DR+ human epidermal antigen presenting cells by in vivo UVA, UVB and UVC irradiation. *J Am Acad Dermatol* 17:792-800, 1987
54. Cooper KD, Breathnach SM, Caughman SW, Palini AG, Waxdal MJ, Katz SI: Fluorescence microscopic and flow cytometric analysis of bone marrow-derived cells in human epidermis: A search for the human analogue of the murine dendritic Thy-1 + epidermal cell. *J Invest Dermatol* 85:546-552, 1985
55. Snyder DS, Wright CL, Ting C: Inhibition of human monocyte antigen presentation, but not HLA-DR expression, by cyclosporine. *Transplantation* 44:407-411, 1987
56. Poulter LW, Janossy G: The involvement of dendritic cells in chronic inflammation. *Scand J Immunol* 21:401, 1985
57. Poulter LW: Antigen presenting cells in situ: Their identification and involvement in immunopathology. *Clin Exp Immunol* 53:513, 1983
58. Nickoloff BJ, Griffiths C: Gamma interferon induces different keratinocyte expression of HLA-DR, DQ and intercellular adhesion molecule-1 antigens (abstr). *J Invest Dermatol* 90:592, 1988

ANNOUNCEMENT

The Departments of Anatomy and Cell Biology, Biological Chemistry, Dermatology, and Environmental & Industrial Health at the University of Michigan, Ann Arbor invite you to attend Recent Developments in Keratinocyte Biology, a symposium honoring Isadore A. Bernstein, Ph.D., Professor of Environmental & Industrial Health, School of Public Health, Professor of Biological Chemistry, The Medical School, The University of Michigan. The event will be held at the University of Michigan, Ann Arbor, Michigan, on Friday and Saturday, May 18th and 19th, 1990 and will feature national and international scientists discussing their recent studies in the area of keratinocyte biology. A dinner in honor of Dr. Bernstein will be held Saturday, May 19th. For further information, call the Office of Development and External Relations, School of Public Health, at 313/764-8093.